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Activity of AMP Against Experimental Herpes Simplex Virus Type 1 Infections in Mice

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Administration of AMP soon after inoculation of mice with herpes simplex virus type 1 inhibited development of virus-induced lesions and appeared to prevent establishment of virus latency. These effects were dependent on both the AMP dose and the time of AMP administration. Regression of herpes simplex virus type 1-induced lesions was also accelerated significantly by AMP treatment in a time- and dose-dependent manner.

The capacity of AMP to prevent both primary and recurrent herpes simplex virus type 1 (HSV-1) infections resulting from inoculation of mouse ear pinnae has been previously reported (1). Here we report the results of therapeutic trials carried out in the same animal model system. These investigations were prompted by several reports indicating possible antiviral activities inherent in adenosine or AMP (5, 10–12), as well as by a growing list of reported pharmacological and physiological effects of adenosine and AMP (2–4, 6, 9).

The mouse ear pinnae model of Hill et al. (7, 8) was utilized as described previously (1). For primary infection, the right ear pinnae of 4-week-old Swiss white mice (CFW Swiss Webster; Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were inoculated subdermally with 25 μl of HSV-1, containing 10^6 PFU. Lesions developing on the pinnae any time within 2 to 7 days post-inoculation were taken as evidence of infection. No attempts were made to scale the intensity of infection, which ranged from macroscopically observable inflammation to vesicle formation. Mice used in reactivation experiments were stressed 3 weeks after healing (approximately 5 weeks after inoculation) by intraperitoneal administration of 5 mg of amphetamine sulfate per kg (1). They were observed for 7 days for development of recurrent lesions of the ear pinnae.

Several HSV-1 strains were initially tested for their capacity to produce lesions in mouse ear pinnae. These strains included LP, F, and MP, obtained from B. Roizman, University of Chicago, Chicago, Ill.; Miyama, obtained from A. Weissbach, Roche Institute of Molecular Biology, Nutley, N.J.; and Bramson, obtained from G. Plummer, Loyola University, Chicago, Ill. Because of its high pathogenicity and capacity to evoke reproducible lesions, HSV-1 LP was used in all experiments. Primary lesions appeared, on the average, 3 to 5 days after inoculation of the ear pinnae with strain LP and persisted for 4 to 5 days thereafter. Recurrent lesions, appearing approximately 48 to 72 h after amphetamine administration, were generally milder and healed more rapidly than primary lesions.

A multiple-dose treatment regimen (four doses over a 24-h period) was used to determine the minimum dose required to completely inhibit the formation of primary lesions. AMP (Sigma Chemical Co., St. Louis, Mo.) was dissolved in phosphate-buffered saline (pH 7.4) just before use and administered intraperitoneally. Nine groups of mice received AMP at 1, 6, 12, and 24 h after inoculation with HSV-1. The individual dose required to reduce the incidence of primary ear lesions by at least 50% was 2.0 mg/kg; the total dose was 8.0 mg/kg (Table 1). The minimum 100% inhibitory dose was 10 mg/kg, or a total of 40 mg/kg over 24 h.

Ten groups of mice inoculated with HSV-1 were used to determine the effectiveness of AMP when treatment was initiated at various times after inoculation. One of these groups served as untreated controls, and nine received four doses of 10 mg of AMP per kg, with treatment initiated at 1, 6, 12, 18, 24, 30, 36, 42, and 48 h after inoculation. Administration of AMP, initiated up to 18 h after inoculation, inhibited 50% or more of the lesions (Table 2).

The effects of AMP therapy on the time required for healing primary HSV-1-induced lesions were studied in five groups of infected mice. In this experiment four of the groups received four doses of 10 mg of AMP per kg in 24 h, with the initial dose administered 24, 48, or 72 h after inoculation or at the first sign of lesion development (inflammation). There was a reduc-

TABLE 1. Dose of AMP required to inhibit primary HSV-1-induced lesions of the ear pinnae

AMP (mg/kg)[a]	No. of mice with lesions/ no. inoculated (%)
0	22/22 (100)
0.05	18/24 (75)
0.20	14/22 (63.6)
0.50	14/22 (63.6)
1.0	15/24 (62.5)
2.0	9/24 (37.5)
5.0	4/20 (20)
10.0	0/20 (0)
20.0	0/20 (0)

[a] The indicated dose of AMP was administered to all mice at 1, 6, 12, and 24 h after infection.

tion in lesion duration in all of the AMP-treated groups, with the most rapid healing occurring in the group first treated 72 h after inoculation (Table 3).

Some of the mice in the above groups were followed to complete regression of the lesions. Three weeks later they were stressed by administration of amphetamine. The greatest reduction in the incidence of recurrent lesions was in the group which first received AMP 72 h after inoculation (Table 3). None of those animals demonstrated evidence of reactivation, suggesting that the previous AMP treatment had prevented the establishment of virus latency.

The results reported here have shown that in the mouse ear pinnae model, AMP is effective in controlling both primary and recurrent HSV-1 infections. AMP was most effective against primary disease when administered within 1 h of virus inoculation, whereas its effect on healing was greatest when treatment was delayed until the onset of lesions. AMP was most effective in inhibiting recurrent infections induced by amphetamine stress when administered either at the time of virus inoculation (data not shown) or just before the primary lesion outbreak. Reasons for the reduced effectiveness or lack of effectiveness of AMP during the incubation period are unknown and will require further investigation and verification. Since we have utilized minimum inhibitory doses of AMP, we may well be able to demonstrate greater effectiveness, even during the apparently refractory incubation period, by increasing the dose. Preliminary studies have shown that mice will tolerate AMP in doses as high as 500 mg/kg. As yet, the effectiveness of AMP against other disease manifestations of HSV-1 (or HSV-2), such as encephalitis or keratitis, has not been tested.

TABLE 2. Influence of the initial time of treatment on the therapeutic efficacy of AMP

Initial time of treatment (h after inoculation)[a]	No. of mice with lesions/ no. inoculated (%)
1	0/10 (0)
6	2/9 (22.2)
12	3/10 (30)
18	5/10 (50)
24	6/10 (60)
30	9/10 (90)
36	9/10 (90)
42	8/10 (80)
48	7/8 (87.5)
Untreated control	10/10 (100)

[a] All mice received 10 mg of AMP per kg at the starting time indicated. Treatment was repeated 6, 12, and 24 h later.

TABLE 3. Effect of AMP on incidence, regression, and recurrence of HSV-1-induced lesions

Initial time of treatment (h after inoculation)[a]	No. of mice with lesions/ no. tested (%)	Mean no. of days required for resolution of lesions[b]	No. of recurrences/ no. stressed (%)
Untreated control[c]	49/49 (100)	4.76	11/26 (42.3)
24	14/26 (53.8)	4.21	5/14 (35.7)
48	22/32 (68.7)	2.63[d]	6/22 (27.2)
72	16/29 (55.1)	1.81[d]	0/16 (0)[e]
At lesion outbreak	18/18 (100)	2.72[d]	Not tested

[a] All mice received 10 mg of AMP per kg at the starting time indicated. Treatment was repeated 6, 12, and 24 h later.

[b] Determined only for those mice positive for primary lesions.

[c] Two groups were utilized, one with 26 and the other with 23 mice. Only the group with 26 mice was utilized in the reactivation experiment.

[d] Compared with the control, $P < 0.001$.

[e] Compared with the control, $P < 0.002$.

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